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EDITORIAL

Gene expression and mental disease[1]

The structure and function of an organ such as the brain depends largely upon its constituent cells and the pattern of proteins which they synthesize; this in turn results from the expression of specific genes within each cell (Milner *et al.* 1987). Given that all human cells contain the same genetic material, differences in cell phenotypes depend upon mechanisms which result in expression or suppression of particular genes and production or absence of particular proteins both during development and in response to stimuli of various kinds. Thus, although recombinant DNA techniques and gene sequencing are of great importance (Baron & Rainer, 1988), the study of normal and abnormal regulation of gene expression and protein synthesis plays a significant complementary role. Of particular relevance to psychiatry are processes occurring in neurons and glia, for example the production of enzymes, transmitters and receptors, and how these processes may be affected in neuropsychiatric disease. The advent of new experimental methods has already led to advances in understanding normal and pathological forms of gene expression in the brain, and their application to psychiatry has rapidly become apparent. We attempt to cover the ground prepared by Owen & Whatley (1988), who concluded their review of molecular genetics in mental disease by stating 'from here we may hope to follow the sequence of changes that finally results in phenotype, so preparing the ground for more rational and effective therapeutic strategies'.

FROM GENE TO PROTEIN

The two-step process by which a gene is first transcribed into messenger RNA (mRNA) followed by translation of the mRNA into a protein is now recognized to consist of multiple smaller steps, each representing a potential site for regulation or defect.

The primary event in expressing a gene is initiation of transcription, which involves alteration in the DNA configuration at the site of the gene, and a number of initiation factors, recognition sequences and enzymes which combine to produce heterogeneous nuclear RNA ('immature' mRNA). Control of this step is not surprisingly under a number of regulatory influences (Darnell, 1982; Lewin, 1985; Ptashne, 1988; Schlief, 1988); the growing mRNA and its termination are also subject to control (Yanofsky, 1988). Newly transcribed nuclear RNA consists of sequences of base pairs coding for the protein, called exons, separated by non-coding sequences, called introns, together with a length of non-coding bases at each end; the non-coding regions are thought to play another regulatory role. Prior to translation, RNA undergoes modification: a methylated cap is added to the 5′ end, and a length of adenosine residues (the 'poly A tail') to the 3′ end. The introns are then excised and the exons spliced together to leave the gene as an uninterrupted linear sequence, which, complete with non-coding ends, cap and tail, is ready to be translated ('mature' mRNA). To complicate the story, for some genes, variations in the position where the introns are excised, a process called alternative splicing, enables one precursor mRNA to generate different mature mRNAs, resulting in more than one protein product (Breitbart *et al.* 1987).

After migration into the cytoplasm and attachment to a ribosome, synthesis of the amino-acid chain begins, involving ribosomal and transfer RNA and another series of regulatory molecules (Lewin, 1985). The peptides may then undergo post-translational modification such as phosphorylation or glycosylation before arriving at their final conformation and destination (Magee & Hanley, 1988). Each mRNA molecule gives rise to a variable number of protein copies prior to degradation by ribonuclease. Messenger RNA half-life depends partly on the poly A tail

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which protects it from this enzyme, and control of the tail may itself be an important regulatory step (Hunt, 1988).

The above processes and constraints appear to occur in all cells. In addition, the brain has a number of tissue-specific mechanisms controlling gene expression (Sutcliffe, 1988). This is made necessary by the dedication of a third of the genome to brain mRNA, and the variety of mRNAs expressed compared to other tissues, reflecting both the overall level of complexity and the differentiation of cell types in the brain. One set of candidates for allowing a gene to be expressed exclusively in the brain are identifier sequences (Milner *et al.* 1987, but see Chikharaishi, 1986). Another brain-specific mechanism is the use of non-adenylated mRNA (poly (A)-mRNA): as mentioned, most mRNAs receive a poly A tail, but the brain contains a population of poly (A)-mRNA, thought to be important in development (Chaudhari & Hahn, 1983). However, technical problems make their identification difficult, and as with other tissue-specific mechanisms they remain poorly understood. This is also true for the ways in which the intracellular processes described above are influenced by external events – a crucial stage in relating molecular biology to clinical psychiatry.

STUDY OF BRAIN GENE EXPRESSION

A corollary of the complexity of brain gene expression is that multiple experimental techniques are needed to investigate it. Moreover, until gaps in understanding the sequence of events from gene to protein are filled, certain assumptions may have to be made as to how changes detected at one point in the process affect others. However, there are now a number of techniques available for investigating different steps on the gene-to-protein pathway which together promise to be of considerable value.

Identification and sequencing of normal and abnormal genes using molecular genetics is providing fundamental information relevant to psychiatric disorders (Owen & Whatley, 1988; Baron & Rainer, 1988). For inherited conditions such as Huntington's chorea this approach is the key to understanding the causation of the disease, and even for multifactorial and polygenic disorders it will be a major advance to locate linked genes which may be of aetiological significance. However, it is unlikely that the function of an encoded protein will be apparent from its gene, and studies of the expression of a gene through to the structure, role and fate of its protein are necessary to help explain how a genetic abnormality results in the clinical picture. While awaiting the identification of linked genes to study, the techniques described below can also be used to investigate how abnormalities in the processing of various genes gives rise to disease pathology. For example, in Alzheimer's disease (AD), alterations in the cholinergic system are unlikely to have a primary causal role (Harrison, 1986) but contribute to the clinical deficits of the disorder (Perry, 1988). Gene expression studies can help clarify the nature of the cholinergic loss and its role in producing the clinicopathological picture of AD. Furthermore, some neuropsychiatric disorders are unlikely to have a major genetic component (e.g. Parkinsonian dementia), and in these cases study of the *expression* of various genes is likely to be the primary focus, rather than a genomic analysis.

Until recently, little was known of the links between genes in the brain and the functioning of brain proteins revealed by neurochemical studies. The advent of several molecular neurobiological techniques is beginning to bridge this gap. Northern blotting uses extracted, purified RNA and separates the different mRNA species by size. Following transfer of the RNA from electrophoretic gel to filter paper, specific mRNAs can be detected by hybridization with a labelled complementary sequence. Patterns of RNA distribution (analagous to the DNA fingerprints made by Southern blotting) are produced and can be compared qualitatively and quantitatively between brain regions or between normal and disease brains (Crapper McLachlan *et al.* 1988). *In vitro* translation tests the functional integrity of mRNA by its ability to give rise to protein, an ability which might be impaired in disease (Gilbert *et al.* 1981; Octave *et al.* 1988). This technique can be used in association with two-dimensional protein electrophoresis to assess the nature and quantity of the resultant protein; differences in translation products in various circumstances can thereby be

investigated (Guillemotte *et al.* 1986; Perrett *et al.* 1988). A third method based on extracted brain mRNA is that of cDNA libraries, whereby an mRNA (and thus an expresssed gene) is used to produce its complementary DNA (cDNA) fragment (Milner *et al.* 1987; Baron & Rainer, 1988). The cDNA can then be sequenced and identified, translated in an appropriate vector, or used as a probe to determine where that particular mRNA is present in another brain region or in a diseased brain (Octave *et al.* 1988). The use of multiple cDNA probes gives a screening method by which mRNAs may be found to be unexpectedly absent (or present) in different situations. Attention can then focus on such mRNAs as representing potential disease associated alterations. This method of 'subtractive cloning' can also be applied to the study of drug-induced effects on gene expression (Friedman *et al.* 1984) and help distinguish these from disease-specific changes.

In contrast to the above techniques, which are limited to regional or whole brain analyses, *in situ* hybridization histochemistry (ISHH) enables gene expression to be investigated at the cellular level, and thus provides a more detailed and localized understanding of gene expression processes. As in Northern blotting, ISHH uses a labelled length of DNA or RNA as a probe to hybridize to, and thus detect, a complementary mRNA strand coding for a particular protein, but within tissue sections (Valentino *et al.* 1987). Cloned cDNAs or their RNA transcription products can be used as probes. Alternatively, synthetic probes can be directed against parts of any gene whose base sequence is known or can be deduced from the amino-acid sequence of its protein. Neuropsychiatric applications of ISHH use 10–20 micron brain sections, and permit gene expression to be studied in individual neurons and glia, and correlated with a number of other parameters. For example, ISHH, which identifies the cell bodies (and possibly dendrites) synthesizing a protein, can be compared to immunocytochemical or autoradiographic localization of that protein. Because proteins may be transported along neuronal processes to distant sites, the two techniques give complementary information: ISHH identifies the cells making an mRNA at a particular time, whereas immunocytochemistry establishes the distribution of the protein product. Comparison of results obtained with these different methods may also yield information on the turnover of individual proteins. ISHH can be related to known morphological features such as cortical laminae or cell types, or to pathological features, for example neurofibrillary tangles and senile plaques in AD, discussed below. ISHH on thin serial sections allows two (or more) mRNAs to be studied in the same cell, and expression of how genes are co-regulated can be investigated. The amount of hybridization in a cell can be determined and is proportional to the level of mRNA present; as a result, quantitative estimates of expression of a gene can be made. However, it is unclear to what extent mRNA levels are an indication of rates and amounts of protein synthesis, the latter being affected by many other factors. As a rule, changes in protein synthetic rates are preceded by an equivalent change in mRNA levels due to altered transcription rates (Marotta *et al.* 1986), but exceptions exist. For example, beta-tubulin synthesis is regulated by changes in the stability of its mRNA (Yen *et al.* 1988), and other genes may also be regulated post-transcriptionally.

On a practical note, mRNA is surprisingly stable post mortem, and permits human autopsy tissue to be used for gene expression studies (Sadjel-Sulkowska & Marotta, 1984; Johnson *et al.* 1986). This stability compares favourably with requirements for neurochemical research (Perry *et al.* 1982), and means that tissue availability need not be a major problem. Conversely, agonal state markedly affects measures of protein synthesis, and must be controlled for (Gilbert *et al.* 1981; Johnson *et al.* 1986; Perrett *et al.* 1988). Other non-specific influences on gene expression which need to be distinguished from those caused directly by a disease include aging and medication (Marotta *et al.* 1986).

Studies of DNA and RNA as outlined above can be combined with established neurochemical and neuropharmacological methods, which in effect are studying the end results of all the preceding processes. Disease-related changes in levels and activities of enzymes and receptors detected by assay, ligand binding and autoradiography, for example, may be due to alterations at any step. It may prove that the primary defect in a particular protein is not with the gene or its expression, but in its post-translational modification or as a result of changes in the cellular environment. Examples of this kind (e.g. increased phosphorylation) will be approached best by standard biochemical

techniques, as genomic and transcriptional analysis will not reveal any abnormality. Conversely, where initial studies suggest the defect lies in the gene itself (e.g. a mutation) or in its transcription (e.g. aberrant splicing), molecular neurobiological techniques will be of particular value. Ultimately, only by investigating at a number of points and by correlations between each approach will specific defects be located, and their many consequences be understood. Advances will also be promoted by technical developments which increase sensitivity and permit other steps on the gene expression pathway to become accessible. Most importantly, sustained and integrated research programmes will be essential given the great molecular complexity of the brain, both in terms of its normal processes and their disruption in disease states.

NEUROPSYCHIATRIC APPLICATIONS

Alzheimer's disease

Although in the long-term psychiatry may benefit from understanding of gene expression in a number of ways, including its role in brain development, the most immediate clinical relevance comes from the study of changes in gene expression as part of the disease process. In this as in other respects, AD is the paradigm condition. This is due both to the availability of tissue from patients and age-matched controls, and because of the known morphological and biochemical changes with which gene expression studies can be correlated. Within AD, amyloid has been the focus of much recent research and is taken as an example of the application of this field to psychiatry.

The amyloid gene became of interest for two reasons. First, amyloid is a key feature of AD, being a major constituent of senile plaques and being deposited around blood vessels. Second, the finding that its gene is located on chromosome 21 (see Owen & Whatley, 1988) fitted in with the occurrence of AD in trisomy 21; this suggested that an abnormality or over-expression of the amyloid gene (such as triplication) might account for the amyloid deposition seen in AD, and that this in turn was a major pathogenic step: precipitation of excess or abnormal amyloid might be the cause for the neuronal degeneration and resultant pathological changes.

The story has not proved to be so simple. The AD gene is now known not to be triplicated in AD (St George Hyslop *et al.* 1987; Tanzi *et al.* 1987; Podlinsky *et al.* 1987) and is probably normal in structure. Attention has therefore switched to defects in its expression to explain the amyloidosis. ISHH studies have shown that expression of the amyloid gene occurs in neurons throughout life and is not restricted either to AD cases or to brain regions affected by AD (Bahmanyar *et al.* 1987; Goedert, 1987); localization of amyloid expression to neurons rather than glia was also confirmed by this work. The implication is that amyloid gene expression may be a necessary, but is not a sufficient, explanation for AD amyloid, with implications for pathogenic theories involving it (Lewis *et al.* 1988). Moreover, total brain amyloid expression is normal in AD, although certain cell populations, such as neurons in the basal nucleus (Cohen *et al.* 1988) and entorhinal cortex (Higgins *et al.* 1988) do show increased amyloid mRNA levels. Hybridization with probes detecting different parts of the amyloid precursor protein mRNA have demonstrated alterations in the splicing of the gene in AD compared to controls, with reduced production of a component which codes for a protease inhibitor (Palmert *et al.* 1988). In consequence, there is current speculation as to the role of increased protease activity in AD pathogenesis (Carrell, 1988). In addition to such transcriptional changes, post-translational modification by excess phosphorylation is also under investigation (Gandy *et al.* 1988). If the defect in AD amyloid is indeed at the mRNA level or beyond, it emphasizes the need to study all points between genotype and phenotype when attempting to account for a particular clinical or pathological feature of a disease. Finally, the amyloid protein is being mapped in the brain using monoclonal antibodies directed against various parts of the molecule, and identified in discrete cell populations (Majocha *et al.* 1988). These can then be related to neuronal groups defined by other parameters such as connectivity (Pearson *et al.* 1985), transmitter type (Bowen, 1988) or antigenic properties (Miller *et al.* 1987).

There are other genes which deserve attention in AD and which could be investigated in a similar fashion to amyloid. Nerve growth factor (NGF) is one example, as it is needed to sustain central

cholinergic neurons (Rennert & Heinrich, 1986), which are heavily affected in AD, and NGF levels decline with normal ageing (Larkfors *et al.* 1987). Second messenger systems, including G proteins and protein kinases are also of interest (Worley *et al.* 1987), as changes therein might be a common mechanism to explain the various transmitter alterations occurring in AD. A final example is the study of structural proteins such as tubulin and tau, in view of the marked involvement of the cytoskeleton in AD (Anderton, 1988). In all cases, interpretation of changes in expression of specific genes must take into account the significant reduction in total mRNA levels and rates of protein synthesis seen in AD (Marotta *et al.* 1986). In this respect, it will be valuable to identify genes whose expression does not change in parallel to the overall decline, but which either falls more precipitously or remains relatively preserved (Crapper McLachlan *et al.* 1988). The overall reduction in gene expression may be due to loss of neurons and reduced demand as well as to impaired transcription and increased RNA degradation (Marotta *et al.* 1986), although the relationship of widespread neuronal sickness to disease pathogenesis is unclear (Mann *et al.* 1981) and is another target for study.

Other mental disorders

The above techniques can also be fruitfully applied to other mental disorders, though as yet there is little data. In schizophrenia and bipolar depression, for example, demonstration of changes in gene expression of monoaminergic enzymes, transmitters or receptors would complement the known neurochemical alterations in these proteins, and help to correlate them with the suspected structural changes in these disorders (Roberts, 1988; Jeste *et al.* 1988). As with AD, variation between cell populations, brain regions and hemispheres, as well as between psychotic and normal brains, deserves investigation. The major practical limitations will be to obtain sufficient brain tissue from a reasonably homogeneous clinical population, and to control for medication status (Reynolds, 1988). As the number of techniques relying on fresh post mortem material grows, together with the value of information gained from them, the development of methods for collection of brains becomes important. These should include detailed prospective evaluation of the patients – including standardized psychopathological and diagnostic descriptions, psychometry and laboratory investigations – to maximize the clinico-pathological correlations which can be made subsequently. Such collaborations will help prevent the compartmentalization of research and ensure that interested molecular biologists are directed towards clinically important problems.

CONCLUSIONS

Investigation of gene expression in the brain is likely to help significantly with the search for pathogenic factors producing psychiatric disease, although such optimism is tempered by the realization of the complexity of the underlying processes. With suitable resources, however, and in combination with the other approaches outlined above, a coherent picture should begin to emerge of the connection between molecular events in a neuron and a clinical syndrome. Molecular genetic methods are identifying the hereditary components of various disorders and at some stage will sequence the gene(s) which may be abnormal. Gene expression research plays a central role linking these and other findings to biochemical studies of the resultant structure and functioning of proteins. As well as helping clarify the pathogenic sequence, such studies may well point to sites of therapeutic manipulation more amenable than DNA-directed approaches and help avoid some of the ethical issues involved in altering the genetic blueprint.

Finally, the focus on molecular neurobiology should in no way preclude holistic medicine (Weatherall, 1987), and established views concerning factors important to mental health are not about to be replaced. Psychiatric problems will continue to result from psychosocial stresses as much as from alterations in the processing of genes, and both will require investigation and intervention. For the time being, however, these experimental approaches seem a profitable avenue to explore towards the common goal of better understanding and treatment of mental disease. Therefore, it is important that clinicians are aware of the technology available in this area and

become involved both in the direction of research and in the assessment and collection of the material in order to maximize the contribution of these techniques to our knowledge of brain disease.

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